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Comparative in vitro study of honey based and silver based wound preparations on cell viability

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ABSTRACT

Background: Since the early 1980s a plethora of dressings has been developed to promote wound healing. The objective of this study was to compare the effects of silver based dressings and honey based dressings on cell viability.

Materials and methods: In this blinded study, keratinocyte cultures were exposed to prepared extracts of each of the following wound dressings for 40 h:

- *Silver based dressings*: Acticoat, Actisorb, Askina, Atrauman-Ag and Contreet.
- *Honey based dressings*: Melladerm gel, Melladerm mesh, Melladerm plus and Mellarsorb.

Controls consisted of cells that were cultured in the same medium, and under the same conditions as those exposed to extracts.

Results: All dressing extracts had an effect on cell viability. Changes in cell morphology from different wound dressing extracts were noted and compared with control groups after 24 h of incubation.

Conclusions: In the silver based extracts group, Atrauman-silver and Acticoat had the most viable cells. For the honey based group, the most viable cells were seen with Melladerm mesh and Mellasorb. There was no significant difference between the best performing silver and honey based wound preparations with regard to cell viability.

1. Background

A large number of dressings were introduced in the 1980s to promote wound healing. The ideal dressing needs to ensure that the wound remains moist, free of infection, toxic chemicals and excess slough. Wound healing consists of debridement, granulation and epithelialization [1].

The epithelialization phase requires an optimum micro-environment and the absence of cytotoxic factors. The choice of dressing is usually made on the basis of personal preference, availability, type, state and site of the wound [2].

For centuries silver has been used as a topical antimicrobial agent to treat wounds and ulcers. Of the large number of silver preparations, silver nitrate is the most widely used in the clinical settings. Silver nitrate has a number of disadvantages including discomfort on application and irritation to tissues. New silver-impregnated dressings were introduced to overcome these limitations [3].

1.1. *Silver*

Silver has been known for its antimicrobial effect against both Gram-positive and -negative microbes including antibiotic resistant strains such as MRSA and streptococci. The antimicrobial effect of Ag^+ is due to four mechanisms. Firstly the silver ion binds to the bacterial cell membrane and thereby

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disrupts its function and impedes various receptors. Secondly, the ion can inhibit the production of ATP by interfering with the bacterial electron transport chain, thus depriving the cell of its energy source. Thirdly the Ag^+ binds to bacterial DNA, thereby inhibiting cell replication. Finally, silver can bind to intracellular building blocks to form insoluble complexes which renders them to be non-functional [4–9].

1.2. Contreet

This hydrocolloid dressing contains a silver complex that is released when wound fluid is absorbed by the dressing.

1.3. Actisorb plus

This dressing consists of a silver impregnated, activated charcoal cloth.

1.4. Acticoat

Consists of a two layered silver coated high density polyethylene mesh, enclosing a single layer of an apertured nonwoven rayon and polyester fabric.

1.5. Askina Calgitrol Ag

This is a technologically advanced wound dressing that incorporates the barrier effectiveness of ionic silver with the absorbency capabilities of calcium alginate and polyurethane foam.

1.6. Atrauman-Ag

This consists of a coarsely woven, water repellent polyamide textile. It is coated with metallic silver which is firmly bound. The fabric is in turn impregnated with a hydrophilic ointment which consists mainly of triglycerides [8,9].

1.7. Honey

The use of honey in the management of wounds was first documented by Egyptians 4000 years ago. It has been reported that the ancient Greeks, Romans and Chinese used honey as a topical antiseptic for sores and skin ulcers. Honey is the substance obtained when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honeybees. It is a supersaturated sugar solution with approximately 17% water [10].

1.7.1. Antimicrobial properties of honey

Antimicrobial activities of honey are related to its high osmolarity, its ability to generate hydrogen peroxide when diluted, its acidity and direct action of antimicrobial chemicals. It also has anti-inflammatory properties [10,11]. Honey promotes debridement and deodourizes wounds. Oxidation of glucose by bacteria yields odourless metabolites such as carbon dioxide and water [12].

Melladerm Gel^{TM} is a gel, containing eco-honey.

Melladerm Plus^{TM} is a yellowish brown ointment, containing eco-honey.

Melladerm Mesh^{TM} is a hydrogel coated dressing on an open weaved polyester gauze containing eco-honey.

Mellasorb^{TM} is an absorbent hydrophilic powder. It contains honey extracts; it is pre-mixed and applied as a hydrogel which can absorb up to 100 ml of fluid.

In order for the cells to survive in a cell culture the following is required: the correct temperature, a good substrate for attachment and the correct culture medium. The medium provides the essential nutrients and growth factors, maintains the correct osmolality and pH and also provides a mean whereby vital gas exchange (CO_2 and O_2) can take place [13].

The toxicity of topical antimicrobial wound dressings is determined by each of the components in the dressing as well as the carrier medium on which the agent is presented to the skin. The objective of this blinded study was to determine and compare the effect on cell viability of honey based (in this case Melladerm^{TM} products) and silver based products currently used in practice.

2. Materials and methods

This was a blinded study whereby each dressing extract was given a code by an individual not involved in the study. Cells were cultured using a Keratinocyte Growth Medium (KGM®-2), a basal medium, from Lonza (Verviers, Belgium). The basal medium was supplemented with a BulletKit® which contained: BPE (bovine pituitary extract), hEGF (human epidermal growth factor), insulin (bovine), hydrocortisone, GA-1000 (Gentamicin, Amphotericin-B), epinephrine, and transferrin. Primary cultures of human keratinocytes were obtained from explants from the foreskins of tested donors. Informed consent was obtained from the parents or guardians.

Primary cultures were allowed to reach confluence. After the second passage a cell count and viability assay (trypan blue) were performed. A uniform volume of 1 ml of the cell suspension (50 000 cells) was seeded into each well of a 24-well plates coated with poly-D-lysine (24-well tissue culture plates were coated with poly-D-lysine 2 h prior to the seeding of the cells). The cells were then incubated for 28 h at 5% CO_2 and 37 °C to allow attachment of the cells to the culture plate.

2.1. Replacing the medium

The culture medium was replaced with 1 ml of the extract for each of the dressings (done in triplicate) and the cells were then cultured for a further 40 h at 37 °C.

2.2. Dressing extracts

2.2.1. Preparation of the extracts

Extracts were prepared as follows: under aseptic conditions all sterile dressings were cut in 1.41 cm × 1.41 cm blocks with care taken not to cross-contaminate any of the products. The wound dressings were then transferred into a 15 ml non-pyrogenic polypropylene centrifuge tube containing 8 ml of medium.

The following dressing extracts were incubated at 5% CO_2 for 24 h at 37 °C: silver dressing used: Acticoat

Table 1 – Silver based dressing extracts[a].

Name	Silver content (mg/100 cm^2)
Acticoat® (Smith&Nephew)	105
Actisorb silver ® (Johnson&Johnson)	2.7
Askina® Calgitrol Ag (B Braun)	Not available
Atrauman-Ag® (Australasia)	35
Contreet® (Coloplast)	85

[a] Data extracted from previously published studies [11].

(Smith&Nephew); Actsorb (Johnson&Johnson); Askina (B Braun); Atrauman-Ag (Hartmann); Contreet (Coloplast) (Table 1) and Honey based Melladerm™ products (Melladerm gel, Melladerm mesh, Melladerm plus and Mellasorb) (Table 2), 8 ml of culture medium containing cells only served as the controls.

2.2.2. *Test conditions*

After a culture period of 24 h the extracts were transferred under aseptic conditions to new centrifuge tubes and relabeled so that the researches were blinded as to which dressings were being tested. The extracts were then incubated for a further 24 h at 5% CO_2 and 37 °C. Controls constituted tubes without dressing extracts.

2.2.3. *MTT assay*

Cell survival was determined by the estimation of mitochondrial competence to reduce MTT and photometry was used to measure absorbance at 450 nm.

2.2.4. *Cell morphology*

Cell morphology was examined by light microscopy after cells were incubated for 40 h and were digitally photographed.

2.2.5. *Statistical analysis*

Quantifiable data was statistically analyzed and any comparison made included the calculation of mean, standard deviations and *P* values (confidence interval of 95%).

3. Results

3.1. *Cell survival (MTT assay)*

Dressing extracts showed variable effects on cell viability including the loss of activity after cells were exposed to different dressing extracts Contreet and Melladerm plus showed the least viable cells. Atrauman-Ag showed an increase in cell activity as compared with controls. Actisorb plus and Melladerm mesh showed less than 5% loss in cell viability.

Table 2 – Honey based dressing extracts.

Name	Honey content (%)
Melladerm Gel™	40
Melladerm Mesh™	20
Melladerm Plus™	49
Mellasorb™	20

3.2. *Cell morphology*

Before the incubation period a confluent monolayer of keratinocytes was noted. The cells had a polygonal shape and were all attached to the multiwell plate (Fig. 1). Cells exposed to extracts of Atrauman-Ag®, Actisorb® and Melladerm Mesh™ varied noticeably in shape and size and contained round to oval nuclei with prominent nucleoli. These cells shared a similar morphology being large and polygonal. Askina® Calgitrol Ag exposed cells showed less variation in cell shape and size. These cells were round to ovoid with less prominent nuclei and nucleoli (Fig. 2). Contreet® and Melladerm Plus™ exposed cells shared similar morphological changes with distinct cytoplasmic projections and a few enlarged cells. These cells also featured less prominent nuclei and nucleoli. Mellasorb™ and Melladerm Gel™ resulted in morphological changes similar to that of Contreet® and Melladerm Plus™ (Fig. 3).

4. Discussion

The results demonstrate that all the dressing extracts had an effect on cell viability.

In the silver based dressings group, Askina Calgitrol Ag and Contreet showed a decrease in cell viability after 40 h of incubation. The presence of calcium alginate in Askina Calgitrol Ag might be a contributing factor as calcium has been reported to have an inhibitory effect on cell proliferation [14]. Actisorb plus and Acticoat showed a loss of cell viability of less than 2%. Atrauman-Ag demonstrated a superior effect with an increased number of viable cells as compared to the control. These findings are consistent with those found in the study comparing Atrauman-Ag with two other products containing silver [14]. It was found that Atrauman-Ag was less toxic to human keratinocytes as compared to the other two dressings. This was attributed to the fact that Atrauman-Ag release fewer silver ions to the surroundings area as compared to the other dressings [15]. The increase in cell viability (more than 100%) was thought to be as a result of an increase in cellular proliferation. Atrauman-Ag is impregnated with a hydrophilic ointment which consists mainly of triglycerides, whether this is a contributing factor could not be established at this point. The different dressings vary in the nature and amount of silver they contain, as found in the study by Paddle-Ledinek [2]. They also differ in the mode of delivery of silver to the wound surface. In order for silver to be biologically active; it must be in a soluble form such as Ag^+ or Ag^0. Ag^+ is the familiar ionic form present in silver nitrate and other ionic silver

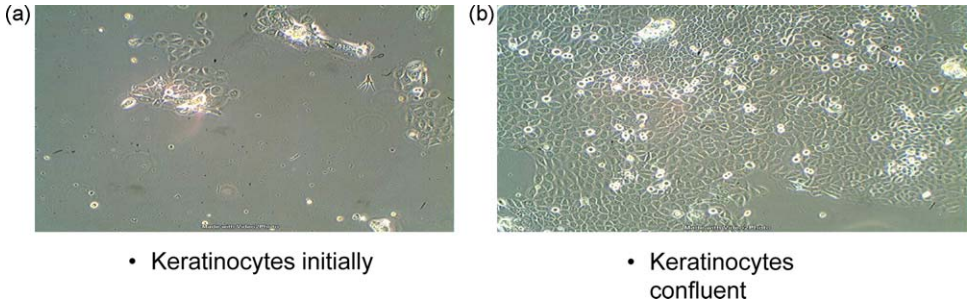

Fig. 1 – (a) Keratinocytes initially and (b) keratinocytes confluent.

compounds. Ag^0 is the uncharged form of silver found in crystalline including nanocrystalline structures. In wound management, silver content should be sufficient to provide sustained bactericidal action [3]. Acticoat with nanocrystalline silver provides the Ag^0 form of silver which is far less rapidly deactivated by chloride or organic matter than the ionic form [3]. Acticoat was found to have less than 30% loss of cell viability which might be attributed to the nature of the silver it contains. Other factors such as the affinity of the dressing for moisture have been implicated in the ability to

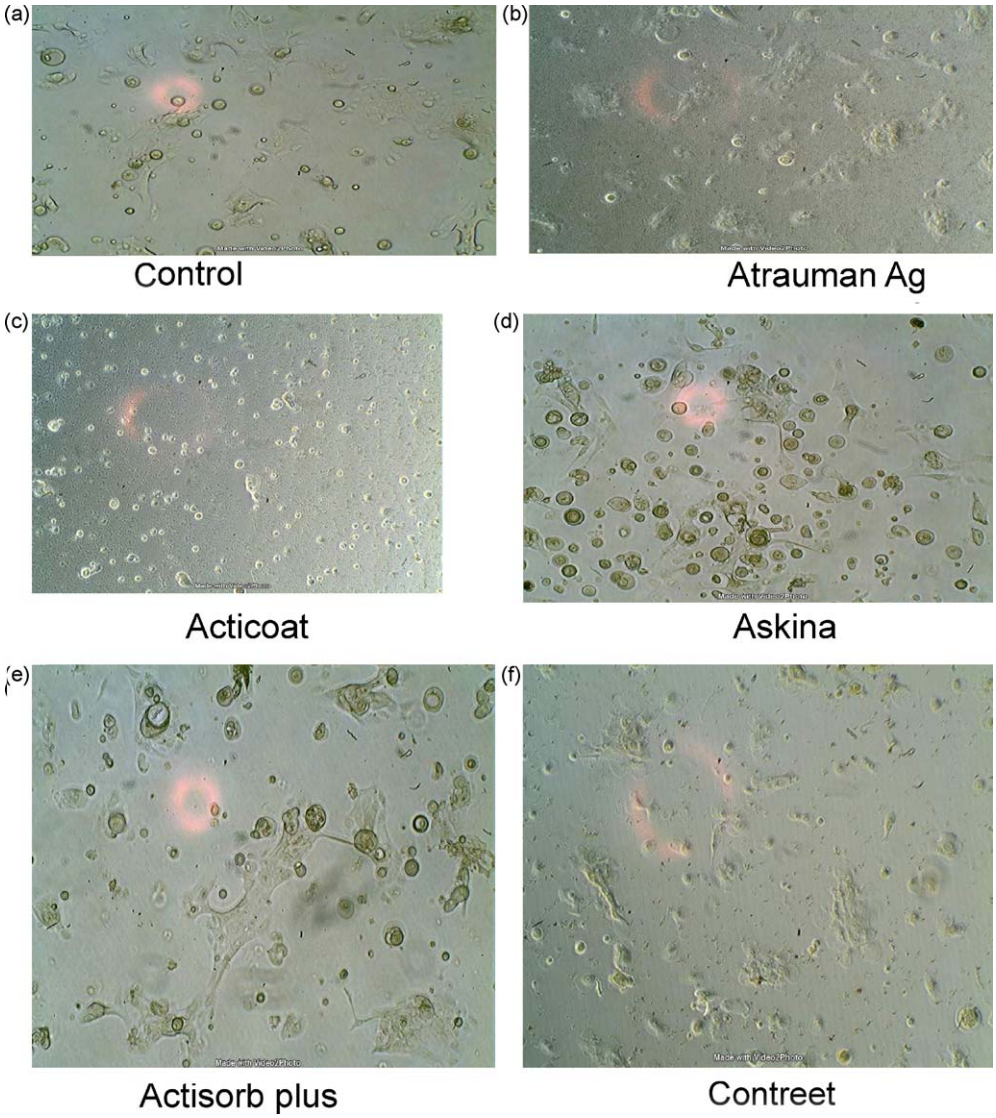

Fig. 2 – Silver wound preparations.

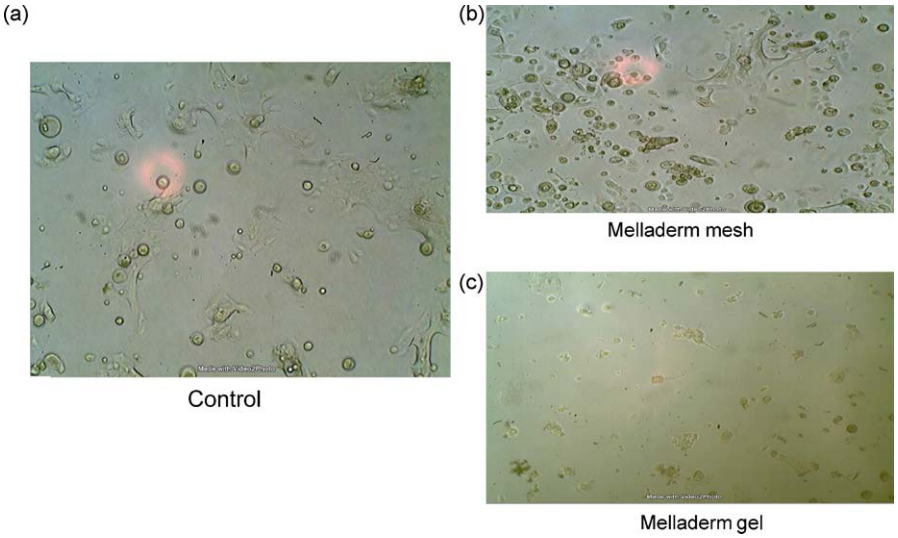

Fig. 3 – Melladerm wound preparations.

kill micro-organisms. This is a prerequisite for the release of active agents in an aqueous environment [12].

In the honey based group, Melladerm mesh exhibited a superior effect with less than 1% loss in cell viability. Melladerm gel and Mellasorb showed a decrease in cell viability of more than 30%. Melladerm plus, performed poorly with loss of cell viability of 40%. This may be attributed to the fact that it is an oil-based dressing and formed an oily layer on the culture medium which may have prevented gaseous exchange and hence loss of cell viability.

Melladerm™ Plus is indicated for all phases of healing and contains several oil products which rapidly promote wound healing. Because Melladerm™ Plus is an oil-based product, when the extracts were introduced to the cell suspension it formed a definite layer on top of the cell suspension. As a result of this visible layer on top of the cell culture suspension no or very little gas exchange could take place between the cells and this environment will consequently kill most of the cells.

Extracts of the various dressings notably influenced cell morphology. Atrauman-Ag®, Actisorb® and Melladerm Mesh™ not only had favorable effects on cell viability, but also showed similar changes in cell morphology after exposure to the extracts. These cells varied in size and shape some being polygonal. The nuclei were round to oval with prominent nucleoli. It appears as if these cells were activated. The other test groups not only showed a decrease in cell number but also featured similar morphological changes with distinct cytoplasmic projections and a few enlarged cells. These cells contained less prominent nuclei and nucleoli.

With any wound dressing a balance must be obtained between antimicrobial efficacy and cytotoxicity [16]. Overall the silver based wound dressings varied with regard to their cytotoxicity in this study. In a study done by Paddle-Ledinek et al. silver based dressings proved to be cytotoxic and were found to induce disordered cell morphology. As a result clinicians were advised not to use silver dressings unless wound infection was a major risk [2]. This was not the case in the present study where the effects of both silver and honey dressing extracts on cell viability showed no significant difference.

One of the main ingredients in the Melladerm™ range of products is honey. Honey has been proven to have an antimicrobial progenies and has been used for this reason since ancient times [17–23]. It has been used for various applications including burn wounds, grafts sites and ulcers to name but a few [18–23]. The healing effect of honey is due to honey's unique properties which include: pH, osmotic effect, high osmolarity, hydrogen peroxide content and some yet to be identified phytochemicals [18–23]. Honey has been shown to be bactericidal to several micro-organisms including *Staphylococcus aureas*, *Klebsiella pneumoniae* and *Proteus mirabilis* amongst others [17–23].

5. Conclusion

Both silver and honey based dressings have an effect on cell viability. In this study we found no significant difference between the best performing silver based wound preparation and the best performing honey based wound preparation.

Declaration of interest

This study was funded by Southern Medical, the manufacturer of Melladerm™ products.

Conflict of interest statement

I Gloria Tshukudu would like to declare that Southern Medical is the manufacturer of Melladerm products. All the necessary steps were implemented to remove the bias.

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